MycoKeys 41: 17–27 (2018)
doi: 10.3897/mycokeys.41.28130
http://mycokeys.pensoft.net

RESEARCH ARTICLE

A peer-reviewed open-access journal
MycoKeys
Launched to accelerate biodiversity research

Description and distribution of *Tuber incognitum* sp. nov. and *Tuber anniae* in the Transmexican Volcanic Belt

Carolina Piña Páez[1], Gregory M. Bonito[2], Gonzalo Guevara-Guerrero[3], Michael A. Castellano[4], Roberto Garibay-Orijel[1], James M. Trappe[4], Rafael Peña Rámirez[3]

1 *Instituto de Biología, Universidad Nacional Autónoma de México, Tercer Circuito s/n, Ciudad Universitaria Delegación Coyoacán, A.P. 70-233, C.P. 04510 Ciudad de México, México* **2** *Department of Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA* **3** *Instituto Tecnológico de Ciudad Victoria. Av. Portes Gil 1301 Poniente, 87010 Ciudad Victoria, Tamaulipas, México* **4** *US Department of Agriculture, Forest Service, Northern Research Station, Forestry Sciences Laboratory, Corvallis, Oregon, 97331, USA*

Corresponding author: *Roberto Garibay-Orijel* (rgaribay@ib.unam.mx)

Academic editor: *K. Hyde* | Received 3 July 2018 | Accepted 12 September 2018 | Published 11 October 2018

Citation: Piña Páez C, Bonito GM, Guevara-Guerrero G, Castellano MA, Garibay-Orijel R, Trappe JM, Rámirez RP (2018) Description and distribution of *Tuber incognitum* sp. nov. and *Tuber anniae* in the Transmexican Volcanic Belt. MycoKeys 41: 17–27. https://doi.org/10.3897/mycokeys.41.28130

Abstract

The genus *Tuber* is a lineage of diverse ectomycorrhizal, hypogeous, sequestrate ascomycete fungi that are native to temperate forests in the Northern Hemisphere. Recently, many new species of *Tuber* have been described in North America and Asia, based on morphological characteristics and molecular data. Here we describe and illustrate a new species, *Tuber incognitum*, based upon phylogenetic analysis and morphological description. We also present a new record for *Tuber anniae* in México. These two *Tuber* species are distributed in the Transmexican Volcanic Belt in the states of México, Michoacán, Guanajuato, Querétaro and Tlaxcala at altitudes between 2,000 and 3,200 meters. These species are associated with *Pinus* (*T. anniae*) and *Quercus* forests (*T. incognitum*).

Keywords

Sequestrate fungi, truffles, Ascomycota, Systematics, new species

Copyright *Carolina Piña Páez et al.* This is an open access article distributed under the terms of the Creative Commons Attribution License (CC BY 4.0), which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.

Introduction

Fungal species, within the genus *Tuber,* produce hypogeous, sequestrate ascomata, that are more commonly known as truffles. These fungi are ectomycorrhizal (EcM) symbionts of angiosperm or gymnosperm hosts, including many species of trees as well as orchids. Plant hosts provide their EcM symbionts with carbohydrates in exchange for greater access to water and nutrients (Wurzburger et al. 2001; Bidartondo et al. 2004; Walker et al. 2005; Shefferson et al. 2008). The genus *Tuber* has been studied intensively over the past century, largely due to its economic importance as an edible fungus. Most of these efforts have been directed towards European species with economic value (e.g. *Tuber melanosporum*, *Tuber magnatum*, *Tuber aestivum*), which reside in a few clades, neglecting most of the diversity in this genus. Reference and environmental sequences data were recently used to infer a minimum of 180–230 species of *Tuber* worldwide (Bonito et al. 2010) and substantiate that most *Tuber* diversity resides within less studied and non-economically important lineages delimitated as the Rufum, Puberulum and Maculatum clades. In México, eighteen *Tuber* species are known and have been formally described. The majority of the collections of these described species are from northeast and central México. In this study, we propose the new species *Tuber incognitum* and provide the first report of *T. anniae* in México based on morphological characteristics and phylogenetic analyses.

Materials and methods

Morphological observation

Ascomata were collected from the states of Guanajuato, México, Michoacán, Querétaro, Tlaxcala and were deposited in herbaria at Oregon State University (OSC), Herbario Nacional de México (MEXU) and Herbario José Castillo Tovar (ITCV). Macroscopic characters were recorded from fresh specimens and microscopic characters were described from both sections of fresh specimens and dried specimens mounted in 5% potassium hydroxide (KOH) following protocols from Castellano et al. (1989).

DNA sequencing and phylogenetic analyses

DNA was extracted from ascomata of collections OSC157842 and OSC150066 using a CTAB chloroform extract protocol and ITS rDNA was amplified and sequenced as previously described (Bonito et al. 2010). Tissue samples from collections MEXU 26504, MEXU 26541, MEXU 26218 and MEXU25995 were sent to the Canadian Center of Barcoding (CCDB) for extraction, amplification, sequencing and barcoding of the Internal Transcribed Spacers (ITS). The ITS region was amplified with ITS1f and ITS4 primers (White et al. 1990). The sequences were edited in Geneious

7.1 (http://www.geneious.com, Kearse et al. 2012). The distribution and ecology of these species was complemented with soil DNA data from central and south México through a BLASTn search against the Mexican Soil Fungi Database in Geneious 7.1. This database includes ITS2 sequences of soil fungi (total DNA soil extractions) from central and southern México as part of an ongoing project, which has been partially published by Argüelles-Moyao and Garibay-Orijel (2018).

DNA sequences were manually trimmed and edited with Sequencher 4.0 (Gene Codes Corp., Ann Arbor, Michigan). ITS sequences were queried against the NCBI public database GenBank by use of the BLASTn algorithm to retrieve similar sequences (Altschul et al. 1990). Collated DNA sequences were aligned with MUSCLE in Mesquite 3.04 (Maddison and Maddison 2015; Edgar 2004). Ambiguously aligned regions were excluded from the alignment. Phylogenetic analyses were conducted on ITS rDNA alignments through the CIPRES portal (www.phylo.org, Miller et al. 2010). Maximum Likelihood (ML) searches were conducted with RAxML v.7.2.8 using rapid bootstrapping of 1,000 pseudoreplicates (Stamatakis 2014). Bayesian Inference (BI) was carried out with MrBayes v3.2.6 (Ronquist and Huelsenbeck 2003). To estimate posterior probabilities, 20,000,000 Markov chain Monte Carlo (MCMC) simulation generations were run in two parallel searches on four chains, with trees sampled every 1,000th generation, with the first 5,000 trees discarded as burn-in (convergence of parallel runs). Bootstrap support, based on 1,000 iterations, was considered informative where it was ≥ 70% and posterior probability was considered significant where it was ≥ 99%. Sequences, generated in this study, are available on GenBank under the accession numbers GQ221447, KC152267, KC152256, KJ595013, KJ595014 and MH174661 (Table 1) and in the BOLD systems database (www.barcodinglife.org).

Results

Descriptions

Tuber incognitum **Piña Páez, Bonito, Guevara & Castellano, sp. nov.**
MycoBank: MB 824931
BOLD systems: ECMTM112, ECMCU046.
Fig. 1a–d

Type. MÉXICO, State of Querétaro, Huimilpan, San Pedro, under *Quercus crassifolia* Humb. and Bonpl., *Quercus* spp., hypogeous, gregarious, 24 September 1996, M.A. Castellano (Holotype: OSC 150066), GB GQ221447. State of Michoacán, Zinapécuaro, el Jaral, under *Quercus polymorpha* Schltdl. and Cham., hypogeous, solitary or in groups of two, 2380 m alt., 19°46'48"N, -100°47'24"W, 4 September 2008, R. Garibay-Orijel (Paratype: MEXU 25995), GB KJ595014. State of México, Temascaltepec, under *Quercus* spp., hypogeous, solitary, 2011 m alt., 19°04'12"N, -100°01'48"W, 8 July 2009, R. Garibay-Orijel (Paratype: MEXU 26218), GB KJ595013.

Table 1. Accession and voucher numbers of sequences generated in this paper.

Taxon	GenBank	Voucher	Origin	Reference
T. incognitum Piña Páez. Bonito, Guevara & Castellano	GQ221447	OSC 150066	Ascoma	This paper
	KJ595013	MEXU 26218	Ascoma	This paper
	KJ595014	MEXU 25995	Ascoma	This paper
	MH174661	–	EcM	This paper
	MH447961	ITCV 1695	Ascoma	This paper
T. anniae W. Colgan & Trappe	MH174660	OSC 157842	Ascoma	This paper
T. bonitoi G. Guevara & Trappe	KC152256	MEXU 26541	Ascoma	Guevara et al. 2015
Tuber sp. 3	KJ152267	MEXU 26504	Ascoma	This paper

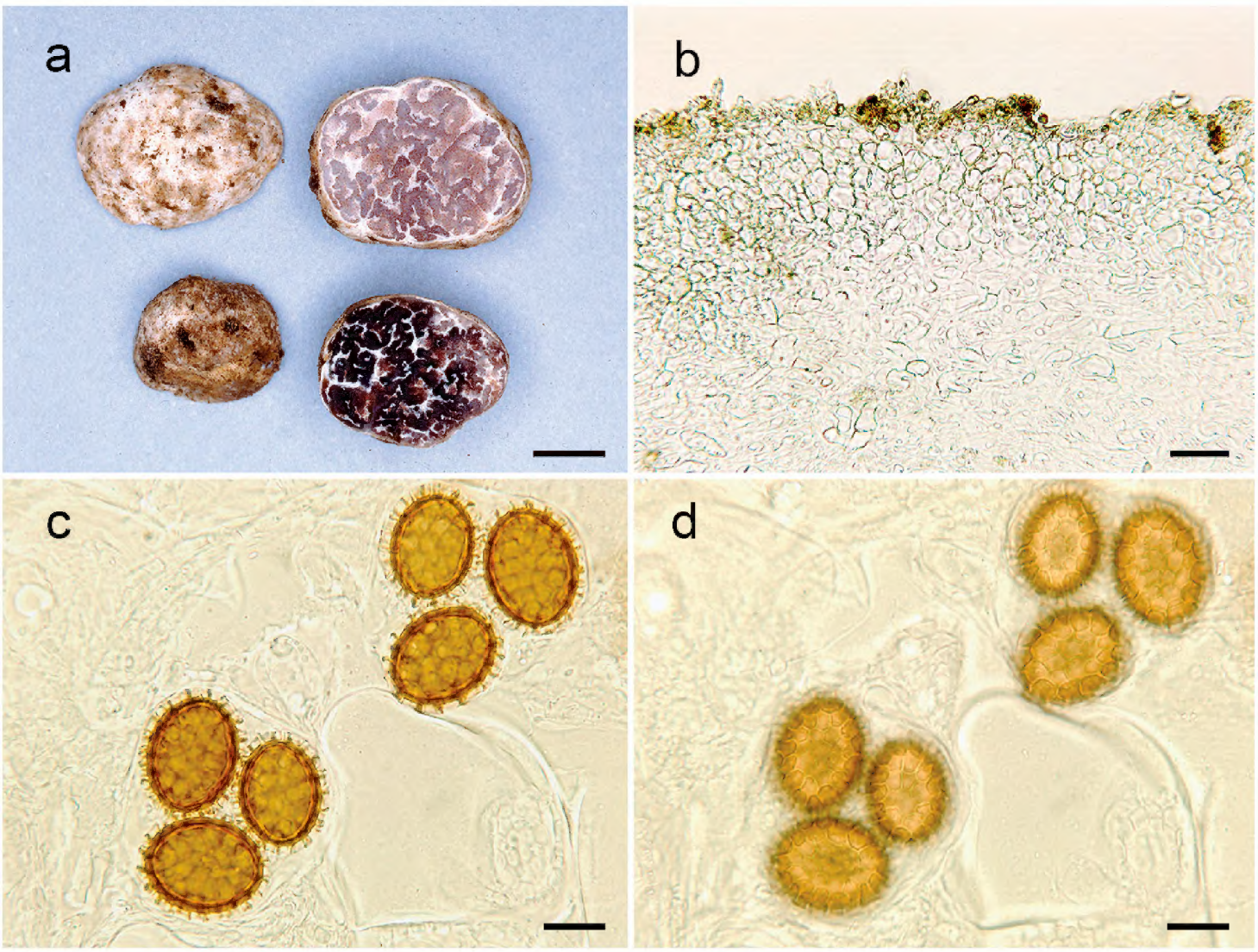

Figure 1. *Tuber incognitum* (Holotype, OSC 150066). **a** Ascoma, surface and cross-section view **b** Peridium in cross-section **c** Light microscopy of spores in cross-sectional view, highlighting the spines and ornamentation **d** Light microscopy of spores in surface view, highlighting the surface and reticulum. Scale bars: 5 mm (**a**), 20 µm (**b**), 15 µm (**c, d**).

Diagnosis. *Tuber incognitum* is distinctive in the structure of its peridium (two-layered) and spore size (25–55 × 20–44 µm), which separates it from the rest of the species within the Puberulum clade reported from México.

Etymology. Incognitum is Latin for unknown. The name incognitum is not derived from its morphology, rather from the fact that it was overlooked for so long. The holotype was collected in 1996 and not described until now.

Description. ***Ascomata*** 10–15 mm broad, subglobose to slightly irregular, white with light brown areas when dry, glabrous, with canals that continue with the veins into the gleba. Gleba pinkish to purplish pale-brown in youth, dark brown at maturity, marbled with white veins. Odour fruity, pleasant.

Peridium two-layered, when handled the upper layer is lost and only the inner layer is observable under the light microscope, 350–400 µm thick, pellis 175–240 µm thick, composed of isodiametric or angular cells, 6–15 µm broad, walls 1.75–2.0 µm thick, yellowish hyaline in KOH. Subpellis 110–140 µm thick, composed of septate, interwoven hyphae (*textura intricata*), 4.5–7.0 µm broad, thin walled < 1 µm thick, hyaline in KOH. Gleba composed of septate, interwoven hyphae (*textura epidermoidea*), 5.0–7.5 µm broad, thin walled < 1 µm thick, hyaline in KOH. ***Ascospores*** broadly ellipsoid; excluding their alveolate-reticulate ornamentation, in 1-spored asci 45–55 × 34–44 µm (Q = 1.3), 2-spored 37–43 × 29–34 µm (Q = 1.25–1.36), 3-spored 30–42 × 26–31 µm (Q = 1.2–1.37), 4-spored 28–33 × 24–28 µm (Q = 1.09–1.25) and 5-spored 25–28 × 20–28 µm (Q = 1.2–1.25), spore colour orange-yellow in KOH, the walls > 2 µm thick; reticulum with 3–8 alveolae across the spore surface; the alveolar walls 3.5–4.0 µm tall. ***Asci*** globose, subglobose to broadly ellipsoid, pyriform, 88–100 × 70–95 µm, pedicel lacking to prominent, hyaline in KOH, hyphae around the asci prostrated or interwoven, cylindrical, 3.5–6.0 µm broad at the septa, thin walled, hyaline in KOH.

Distribution and ecology. Only known from central and southwest México (Querétaro, Michoacán, State of México, Guanajuato and Hidalgo). Ascocarps always associated with *Quercus* species (*Q. crassifolia*, *Q. polymorpha*). An EcM association with *Quercus* has been verified (MH174661) and its DNA has been recovered only from soil in *Quercus* forest in Hidalgo, México.

Additional collections examined. MÉXICO, State of Guanajuato, Guanajuato, Las Palomas, under *Quercus* spp., hypogeous, in groups of two, 2534 m alt., 21°03'50"N, -101°13'23"W, 10 October 2016, R. Peña-Ramirez (ITCV 1695).

Taxonomic comments. *Tuber incognitum* resembles *Tuber pseudoseparans* in the colour of the peridium and the lack of dermatocystidia but differs by the size of the spores (being smaller in *T. incognitum*, 31–50 × 24–37 µm vs. *T. pseudoseparans*, 46–65 × 34–46 µm) and in the thickness of the peridium (being thinner in *T. pseudoseparans*, by ± 250 µm). *Tuber incognitum* is similar to *Tuber bonitoi* in spore size and ornamentation, but differs by the presence of dermatocystidia, which are absent in *T. incognitum* and the thickness of the peridium, being thicker in *T. bonitoi* (200–500 µm). *Tuber incognitum* is similar to *Tuber guzmanii* in the peridial organisation, both species have a well differentiated two-layered peridium but differ in the thickness (being thinner in *T. guzmanii*, 100–160 µm) and spore ornamentation (alveolate reticulum, 2–4 µm tall) and the size of the spores (being larger in *T. guzmanii*, 27–68 × 30–50 µm). The collection from Guanajuato represents a young developmental stage of *T. incognitum*, this collection has a thinner peridium (130–345 µm) and smaller spores (1-spored asci 23–35 × 19–25 µm, Q = 1.09 1.59; 2-spored 18–29 × 17–22 µm, Q = 1.0–1.61; 3-spored 30–42 × 26–31 µm, Q = 1.11–1.2; 4-spored 23–27 × 19–25 µm, Q = 1.08–1.26). These differences represent morphological variation within the species and its identity was confirmed with molecular data.

***Tuber anniae* W. Colgan & Trappe**
Fig. 2a–d

Description. ***Ascomata*** subglobose to slightly irregular, 10–12 mm broad, white, cream, light brown when dry. Peridium thin, < 0.2 mm, smooth to velvety, irregularly roughened, furrows with depressions continuing as canals into the gleba. Gleba solid, brown, marbled with white veins that emerge as depressions on the peridium. Odour and taste not recorded.

Peridium 85–140 µm thick; pellis a pseudoparenchyma, 40–65 µm thick, cells 6–18 µm broad, versiform, isodiametric, squared, rectangular or angular, hyaline to yellowish in KOH, thick walled (> 1.0 µm), dermatocystidia absent; subpellis 45–75 µm thick, of hyaline, septate, interwoven hyphae (*textura epidermoidea*), 4.0–5.5 µm broad, thin walled, < 1 µm thick. Gleba of hyaline, interwoven, sinuous hyphae, 5.0–7.5 µm broad, constrained at the septum, 3.0–4.5 µm broad at the septa, thin-walled (< 1.0 µm).

Ascospores subglobose; excluding their alveolate-reticulate ornamentation, 1-spored asci 40–50 × 30–46 µm (Q= 1.03–1.15), 2-spored 28–38 × 26–35 µm (Q = 1.05–1.13), 3-spored 26–33 × 24–30 µm (Q = 1.04–1.15), spore colour orange-yellowish in KOH; walls > 2 µm thick, yellow; reticulum with 5–6 aveolae across the spore surface; the alveolar walls 3–4.5 µm tall. ***Asci*** subglobose, 84–105 × 75–85 µm, pedicel lacking to prominent, walls with 2–3 layers, hyaline in KOH; hyphae around the asci interwoven, 3.5–5.5 µm broad at the septum, thin walled (< 1.0 µm), hyaline in KOH.

Distribution and ecology. Wang et al. (2013) reported from Europe; Colgan and Trappe 1997; Bonito et al. (2010) reported in North America. Here, we extended the distribution to central México (State of México and Tlaxcala). In Finland, *T. anniae* has been confirmed to establish association with *P. sylvestris* L. (Wang et al. 2013). In Washington, this species has been confirmed to establish association with *Pseudotsuga menziesii* (Mirbel) Franco (Bonito et al. 2010). In México, sporocarps always collected co-occurring with *Pinus leiophylla* Schiede and Deppe and *Abies religiosa* (Kuntch) Schldl. and Cham. In México, the only environmental DNA of this species has been recovered from soil in conifer forests in Tlaxcala associated with *Pinus montezumae* and in State of México associated with *A. religiosa* (Argüelles-Moyao and Garibay-Orijel 2018).

Collections examined. MÉXICO, State of Tlaxcala, Huamantla, cañada central, La Malinche National Park, under *Pinus leiophylla* Schiede and Deppe and *Abies religiosa* (Kunth) Schltdl. and Cham., hypogeous, solitary, 3220 m alt., 19°14'7"N, -97°59'9"W, 23 September 2007, G.M. Bonito (OSC 157842), GB MH174660.

Taxonomic comments. *Tuber anniae* is similar to *Tuber pacificum* Trappe, Castellano and Bushnell, however, the latter species has narrower, ellipsoid spores (23–15 × 16–35 µm) and a thicker peridium (250–400 µm) than the former. *T. pacificum* has also been found co-occurring with *Pseudotsuga menziesii* and *Tsuga heterophylla* (Raf.) Sarg. along costal Oregon, while *T. anniae* has been found co-occurring with *P. leiophylla* and *A. religiosa.*

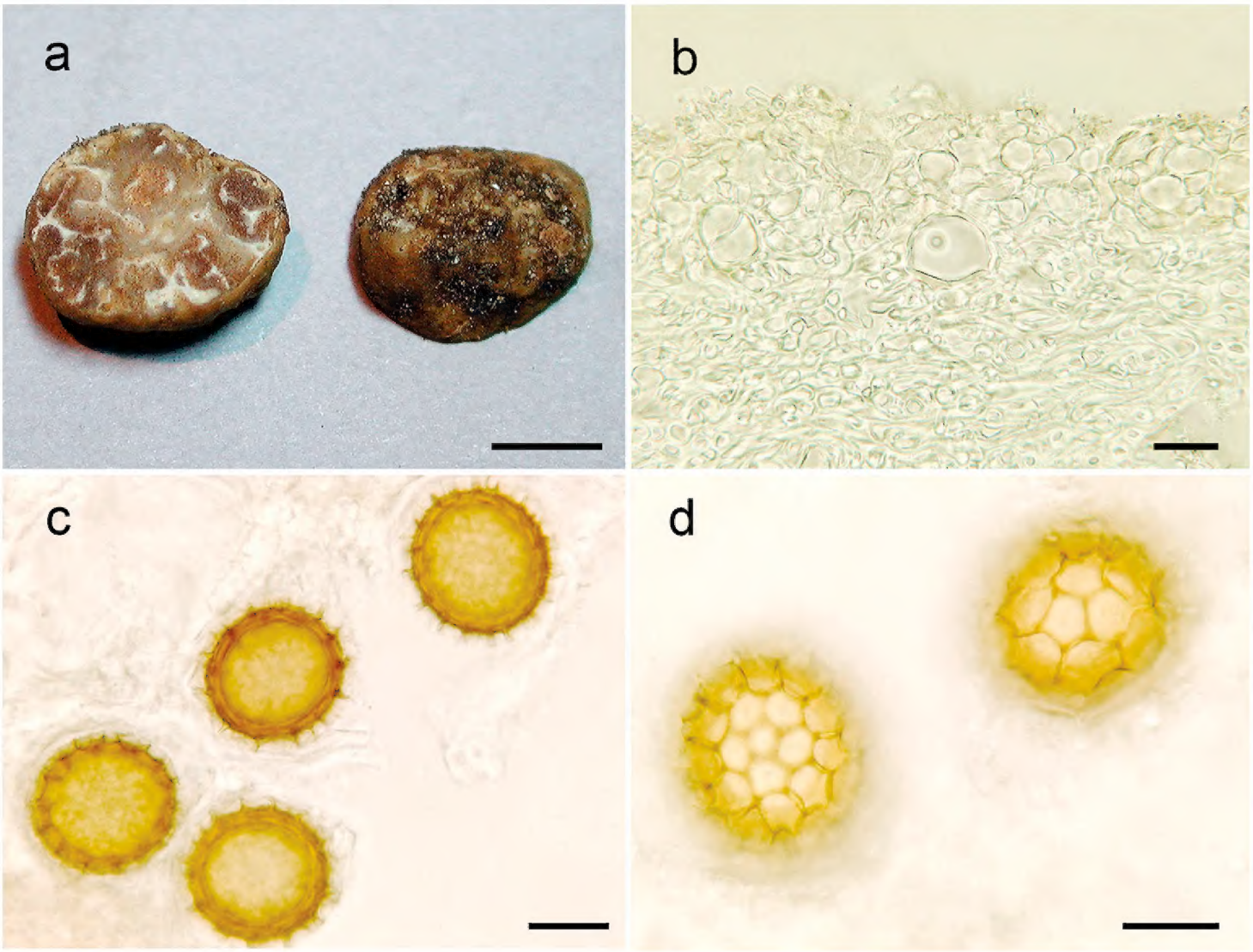

Figure 2. *Tuber anniae* (OSC 157842). **a** Ascoma, surface and cross-section view **b** Peridium in cross-section **c** Light microscopy of spores in cross-sectional view, highlighting the spines and ornamentation **d** Light microscopy of spores in surface view, highlighting the surface and reticulum. Scale bars: 5 mm (**a**), 15 µm (**b, c, d**).

Tuber anniae was first described by Colgan and Trappe (1997). The holotype (from Washington) and the other collections reported were from the Pacific Northwest in the US and reported co-occurring with *P. menziesii*. The *T. anniae* complex of species has been proposed based on phylogenetic analysis using ITS region (Wang et al. 2013). The collection from México is very similar to the holotype collection, however, the latter has a brown to dark olive-brown peridium and its spores have thicker (up to 5 µm) spore walls than the former. Additionally, *T. anniae,* as described by Colgan and Trappe (1997), has mostly globose spores with 10–16 alveolae across the spores. The Finnish collections exhibit subtle morphological differences in comparison with the collections from North America. The Finnish specimens have a smooth peridial surface, except along the grooves and around the pits (Wang et al. 2013), while the holotype specimen was reported to be smooth and lack dermatocystidia (Colgan and Trappe 1997). It seems that the presence of dermatocystida only along the grooves and/or at the bottom of pits in *Tuber* collections is likely the result of handling of the ascoma during processing (Dr. D. Luoma, personal communication). Additionally, the spores from the Finnish specimens have larger dimensions (27–60 × 27–56 µm; Q = (1.00) 1.05–1.20 (1.33)) than the specimens described for *T. anniae* by Colgan and Trappe from Washington (1997).

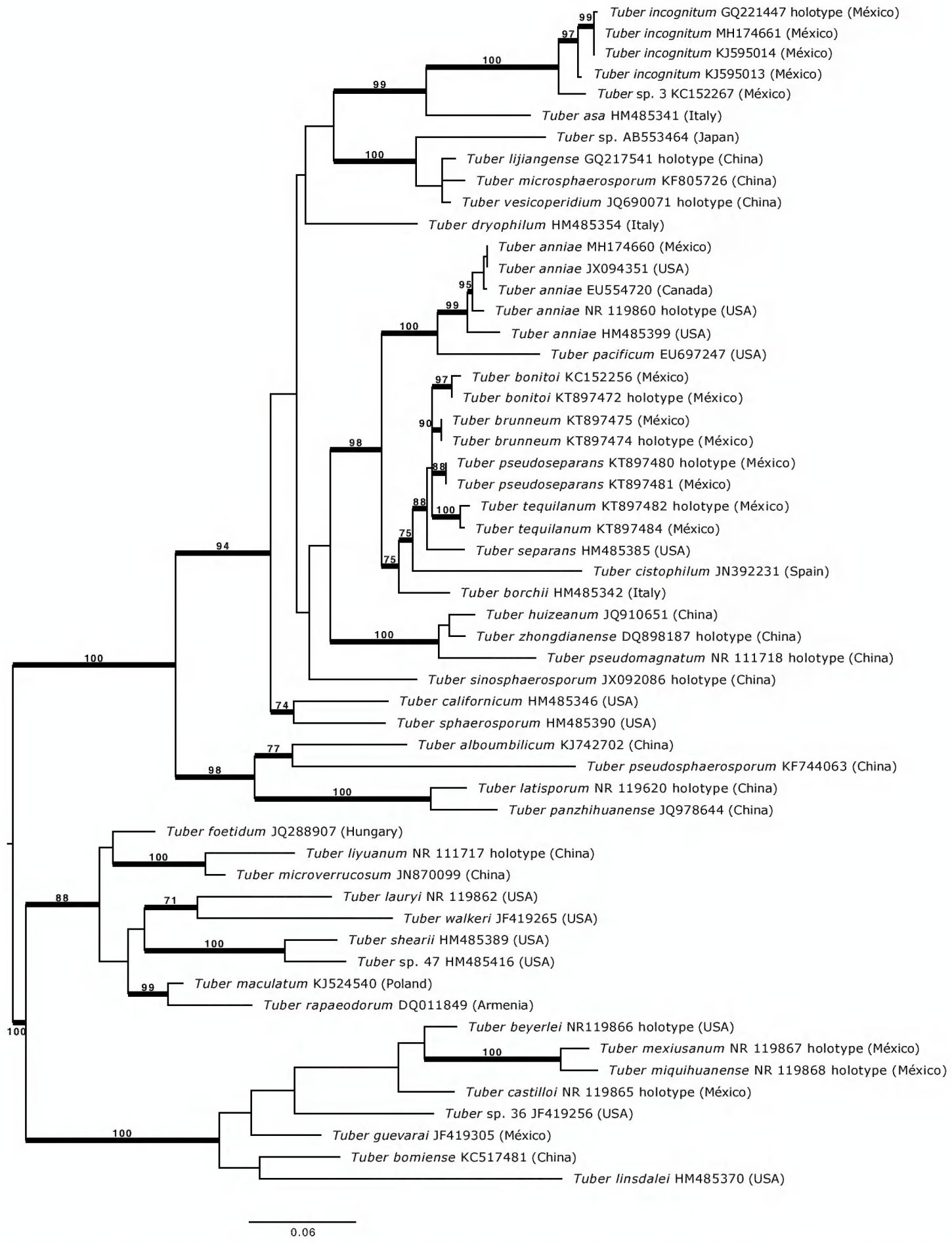
Tuber incognitum GQ221447 holotype (México)
Tuber incognitum MH174661 (México)
Tuber incognitum KJ595014 (México)
Tuber incognitum KJ595013 (México)
Tuber sp. 3 KC152267 (México)
Tuber asa HM485341 (Italy)
Tuber sp. AB553464 (Japan)
Tuber lijiangense GQ217541 holotype (China)
Tuber microsphaerosporum KF805726 (China)
Tuber vesicoperidium JQ690071 holotype (China)
Tuber dryophilum HM485354 (Italy)
Tuber anniae MH174660 (México)
Tuber anniae JX094351 (USA)
Tuber anniae EU554720 (Canada)
Tuber anniae NR 119860 holotype (USA)
Tuber anniae HM485399 (USA)
Tuber pacificum EU697247 (USA)
Tuber bonitoi KC152256 (México)
Tuber bonitoi KT897472 holotype (México)
Tuber brunneum KT897475 (México)
Tuber brunneum KT897474 holotype (México)
Tuber pseudoseparans KT897480 holotype (México)
Tuber pseudoseparans KT897481 (México)
Tuber tequilanum KT897482 holotype (México)
Tuber tequilanum KT897484 (México)
Tuber separans HM485385 (USA)
Tuber cistophilum JN392231 (Spain)
Tuber borchii HM485342 (Italy)
Tuber huizeanum JQ910651 (China)
Tuber zhongdianense DQ898187 holotype (China)
Tuber pseudomagnatum NR 111718 holotype (China)
Tuber sinosphaerosporum JX092086 holotype (China)
Tuber californicum HM485346 (USA)
Tuber sphaerosporum HM485390 (USA)
Tuber alboumbilicum KJ742702 (China)
Tuber pseudosphaerosporum KF744063 (China)
Tuber latisporum NR 119620 holotype (China)
Tuber panzhihuanense JQ978644 (China)
Tuber foetidum JQ288907 (Hungary)
Tuber liyuanum NR 111717 holotype (China)
Tuber microverrucosum JN870099 (China)
Tuber lauryi NR 119862 (USA)
Tuber walkeri JF419265 (USA)
Tuber shearii HM485389 (USA)
Tuber sp. 47 HM485416 (USA)
Tuber maculatum KJ524540 (Poland)
Tuber rapaeodorum DQ011849 (Armenia)
Tuber beyerlei NR119866 holotype (USA)
Tuber mexiusanum NR 119867 holotype (México)
Tuber miquihuanense NR 119868 holotype (México)
Tuber castilloi NR 119865 holotype (México)
Tuber sp. 36 JF419256 (USA)
Tuber guevarai JF419305 (México)
Tuber bomiense KC517481 (China)
Tuber linsdalei HM485370 (USA)
0.06

Figure 3. Most likely tree based on maximum likelihood phylogenetic inference showing the placement of *Tuber incognitum* within the Puberulum clade. Bootstrap values ≥ 70% are labelled above nodes. Nodes with posterior probabilities ≥ 99% are blacked. Holotype collections are labelled. The phylogeny is rooted with species belonging to the Maculatum clade. Scale bar corresponds to the mean number of nucleotides substitutions per site.

Discussion

Species in Puberulum clade can be found in North America, Europe and Asia and some regions of North Africa and South America (Bonito et al. 2010, 2013; Jeandroz et al. 2008; Payen et al. 2014). There are some records of species in the Puberulum clade (e.g. *Tuber rapaeodorum*) that have been introduced into Australia and New Zealand (Bonito et al. 2010). Species in this clade show a wider range of host associations than other species within the Rufum, Excavatum, Aestivum, Maculatum and Gennadii clades (Payen et al. 2014). Species within the Puberulum clade commonly form associations with angiosperms and conifers (Bidartondo et al. 2004; Bonito et al. 2010). In México, twenty species of *Tuber* have been reported, including the two species from this study. Eight of the twenty belong to the Puberulum clade. Both the Maximum Likelihood and Bayesian analyses (Figure 3) show that *T. incognitum* forms a strongly supported clade (Maximum Likelihood bootstrap= 97), which includes sequences from both voucher collections and EcM root tip collections. This clade is placed as a sister taxon of the undescribed species *Tuber* sp. 3 (KC152267) also from México. This study combines sporocarp anatomy, molecular analyses and phylogenetic analyses to support the erection of *T. incognitum* as a unique species within the Puberulum clade.

The *T. anniae* species complex is recovered as a strongly supported clade. There is an internal structure in this clade, with different branch lengths and nested subclades, but additional markers are needed to resolve relationships within this species complex. The Mexican specimens' group with those from Alaska (JX094351), form a nested clade that is closely related to a collection from Canada (EU554720). The members in the *T. anniae* species complex are closely related to *T. pacificum* from Oregon, USA. Given the relatively high ITS similarity, phylogenetic position and similar morphology to the *T. anniae* holotype collection, we have identified the Mexican collection as *T. anniae,* extending its known range and southernmost distribution of this species in North America.

Acknowledgements

This research was made possible through NSF award number 0641297, REVSYS: Phylogenetic and Revisionary Systematics of North American Truffles (*Tuber*). GB thanks AgBioResearch MICL02416 for research support. DNA sequencing was supported by the MEXBOL network project CONACYT 280896. CPP thanks the North American Truffling Society for research support, Daniel Luoma, Javier Tabima and Antonio Gómez who provided insight and expertise that greatly assisted the research. GG thanks CONACYT and TecNM for research support. We thank the herbaria of ITCV, MEXU and OSC for accessing their collections.

References

Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ (1990) Basic local alignment search tool. Journal of Molecular Biology 215(3): 403–10. http://dx.doi.org/10.1016/S0022-2836(05)80360-2

Argüelles-Moyao A, Garibay-Orijel R (2018) Ectomycorrhizal fungal communities in high mountain conifer forests in central México and their potential use in the assisted migration of Abies religiosa. Mycorrhiza 28: 509. http://dx.doi.org/10.1007/s00572-018-0841-0

Bidartondo MI, Burghardt B, Gebauer G, Bruns TD, Read DJ (2004) Changing partners in the dark: isotopic and molecular evidence of ectomycorrhizal liaisons between forest orchids and trees. Proceedings of the Royal Society of London B: Biological Sciences 271(1550): 1799–806. http://dx.doi.org/10.1098/rspb.2004.2807

Bonito GM, Gryganskyi AP, Trappe JM, Vilgalys R (2010) A global meta-analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. Molecular Ecology 19(22): 4994–5008. https://doi.org/10.1111/j.1365-294X.2010.04855.x

Bonito G, Smith ME, Nowak M, Healy RA, Guevara G, Cázares E, Kinoshita A, Nouhra ER, Domínguez LS, Tedersoo L, Murat C (2013) Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified southern hemisphere sister lineage. PloS One 8(1): e52765. https://doi.org/10.1371/journal.pone.0052765

Castellano MA, Trappe JM, Maser C (1989) Key to spores of the genera of hypogeous fungi of north temperate forests with special reference to animal mycophagy. Mad River Press, 186 pp.

Colgan III W, Trappe JM (1997) NATS truffle and truffle-like fungi. 7. *Tuber anniae* sp. nov. (Ascomycota). Mycotaxon 64: 437–442.

Edgar RC (2004) MUSCLE: multiple sequence alignment with high accuracy and high throughput. Nucleic Acids Research 32(5): 1792–1797. http://dx.doi.org/10.1093/nar/gkh340

Guevara G, Bonito G, Cázares-González E, Healy R, Vilgalys R, Trappe JM (2015) Novel *Tuber* spp. (Tuberaceae, Pezizales) in the Puberulum group from México. Ascomycete.org 7(6): 367–74. https://doi.org/10.25664/art-0161

Jeandroz S, Murat C, Wang Y, Bonfante P, Tacon FL (2008) Molecular phylogeny and historical biogeography of the genus *Tuber*, the 'true truffles'. Journal of Biogeography 35(5): 815–29. https://doi.org/10.1111/j.1365-2699.2007.01851.x

Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T (2012) Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28(12): 1647–9. http://dx.doi.org/10.1093/bioinformatics/bts199

Maddison WP, Maddison DR (2015) Mesquite: a modular system for evolutionary analysis. http://mesquiteproject.org [Version 3.04]

Miller MA, Pfeiffer W, Schwartz T (2010) Creating the CIPRES Science Gateway for inference of large phylogenetic trees. Gateway Computing Environments Workshop (GCE): 14 Nov 2010, New Orleans, 1–8. http://dx.doi.org/10.1109/GCE.2010.5676129

Payen T, Murat C, Bonito G (2014) Truffle phylogenomics: new insights into truffle evolution and truffle life cycle. Advances in Botanical Research 70: 211–234. http://dx.doi.org/10.1016/B978-0-12-397940-7.00007-0

Ronquist F, Huelsenbeck JP (2003) MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19(12): 1572–4. http://dx.doi.org/10.1093/bioinformatics/btg180

Shefferson RP, Kull T, Tali K (2008) Mycorrhizal interactions of orchids colonizing Estonian mine tailings hills. American Journal of Botany 95(2): 156–64. http://dx.doi.org/10.3732/ajb.95.2.156

Stamatakis A (2014) RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. Bioinformatics 30(9): 1312–3. http://dx.doi.org/10.1093/bioinformatics/btu033

Wang XH, Benucci GM, Xie XD, Bonito G, Leisola M, Liu PG, Shamekh S (2013) Morphological, mycorrhizal and molecular characterization of Finnish truffles belonging to the *Tuber anniae* species-complex. Fungal Ecology 6(4): 269–80. http://dx.doi.org/10.1016/j.funeco.2013.03.002

Walker JF, K Miller OR, Horton JL (2005) Hyperdiversity of ectomycorrhizal fungus assemblages on oak seedlings in mixed forests in the southern Appalachian Mountains. Molecular Ecology 14(3): 829–38. http://dx.doi.org/10.1111/j.1365-294X.2005.02455.x

White TJ, Bruns T, Lee SJ, Taylor JL (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. PCR Protocols: A Guide to Methods and Applications 18(1): 315–22. http://dx.doi.org/10.1016/B978-0-12-372180-8.50042-1

Wurzburger N, Bidartondo MI, Bledsoe CS (2001) Characterization of *Pinus* ectomycorrhizas from mixed conifer and pygmy forests using morphotyping and molecular methods. Canadian Journal of Botany 79(10): 1211–6. http://dx.doi.org/10.1139/b01-079